Enhanced latent inhibition in dopamine receptor-deficient mice is sex-specific for the D$_1$ but not D$_2$ receptor subtype: implications for antipsychotic drug action

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Abstract

Latent inhibition (LI) is reduced learning to a stimulus that has previously been experienced without consequence. It is an important model of abnormal allocation of salience to irrelevant information in patients with schizophrenia. In rodents LI is abolished by psychotomimetic drugs and in experimental conditions where LI is low in controls, its expression is enhanced by antipsychotic drugs with activity at dopamine (DA) receptors. It is however unclear what the independent contributions of DA receptor subtypes are to these effects. This study therefore examined LI in congeneric DA D$_1$ and D$_2$ receptor knockout (D$_1$ KO and D$_2$ KO) mice. Conditioned suppression of drinking was used as the measure of learning in the LI procedure. Both male and female DA D$_2$ KO mice showed clear enhancement of LI reproducing antipsychotic drug effects in the model. Unexpectedly, enhancement was also seen in D$_1$ KO female mice but not in D$_1$ KO male mice. This sex-specific pattern was not replicated in locomotor or motor coordination tasks nor in the effect of DA KOs on baseline learning in control groups indicating some specificity of the effect to LI. These data suggest that the dopaminergic mechanism underlying LI potentiation and possibly antipsychotic action may differ between the sexes, being mediated by D$_2$ receptors in males but by both D$_1$ and D$_2$ receptors in females. These data suggest that the DA D$_1$ receptor may prove an important target for understanding sex differences in the mechanisms of action of antipsychotic drugs and in the aetiology of aberrant salience allocation in schizophrenia.

Key words: Antipsychotic, dopamine D$_1$ receptor, dopamine D$_2$ receptor, dopamine knockout mice, latent inhibition, sex difference.

Introduction

Latent inhibition (LI) is a process of learning to ignore irrelevant stimuli and is manifested as slower learning about a stimulus that has been pre-exposed without consequence (PE), compared to learning about the same stimulus without pre-exposure (NPE) (Lubow and Moore, 1959). LI is used to model abnormalities in information filtering in schizophrenia, where patients have difficulty selecting between relevant and irrelevant information (Barak and Weiner, 2007; Gray et al., 1997; Kapur et al., 2005; Lubow, 2005; Weiner, 2003). LI is disrupted in acute schizophrenia patients, their relatives and high schizotypal individuals, and normalized by antipsychotic drug treatment in patients (Baruch et al., 1988; Cohen et al., 2004; Gray et al., 1991; Lubow, 2005; Rascl et al., 2001). LI shows parallel pharmacological profiles in animals and humans (Moser et al., 2000; Weiner, 2003). In rodents, psychotomimetic drugs such as amphetamine impair LI, while antipsychotic drugs enhance low levels of LI produced by decreasing the amount of pre-exposure (Moser et al., 1996; Moser et al., 2000; Palsson et al., 2003).
There is increasing evidence for a differential contribution of DA release to LI (considered to be mediated by nucleus accumbens DA release), is reversed by the D₂ receptor antagonists on low LI are reproduced in mice that have genes for D₁ or D₂ receptors deleted independently and whether D₁ or D₂ receptors are independently essential for the normal expression of (high) LI. LI was investigated using two levels of pre-exposure, one producing LI in wild-type (WT) animals (60 PE) and one not (40 PE). This facilitates the observation of potential enhancement or reduction of LI. To assess consistency with prior behavioural studies and relevance to motor side-effects of antipsychotic drugs, locomotor activity and motor coordination on the rotorod were also assessed.

Materials and methods

Animals

The original F₂ hybrid strain (129/Sv × C57BL/6J) containing the mutated D₁ or D₂ receptor alleles were generated as reported previously (Drago et al., 1994; Kelly et al., 1997). The targeted gene deletion was constructed in 129/Sv embryonic stem cells and male chimaeras mated with C57BL/6 females to produce heterozygous mutants (D₁⁺/− or D₂⁺/−). Incipient congenic D₁ and D₂ lines were established by backcrossing heterozygous mutants to WT C57BL/6 for 14 generations. Homozygous KO mice (D₁⁻/- and D₂⁻/-) and WT (D₁⁺/+ and D₂⁺/+)) littermates were bred by heterozygous intermatings of the incipient congenic heterozygote mutants imported from Royal College of Surgeons, Dublin, Ireland. Congenic strains have significant advantages over mixed background strains in reducing inter-animal variability and maximizing the specificity of the phenotype to the targeted gene (Gerlai, 1996; Silva et al., 1997). D₁ KO mice had low body weight and poor growth and were weaned later than D₂ KO mice to facilitate growth and survival. They were given a wet mash diet in the home cages after weaning, this has been reported previously in these mice (Doherty et al., 2008). D₁ KO mice were bred with the kind permission of Professor John Drago, University of Melbourne. D₂ KO mice were bred with the kind permission of Professor Malcolm Low, Oregon Health and Science University, USA.

Male and female KO mice and WT littermates aged at least 10 wk were used in the experiments. Animals were housed 1–4 per cage under a 12-h light–dark cycle (lights on 07:00 hours), at constant temperature and humidity with food available ad libitum.
Experiments were performed during the light period. Mice used for the suppression of licking model were subjected to water restriction periods of 23 h. All experiments were performed in accordance with local and national rules on animal experimentation, and with appropriate personal and project licence authority under the Animals (Scientific Procedures) Act, UK 1986 (PPL no: 40/2883).

Genotyping

Genotyping was performed by PCR using genomic DNA extracted from ear biopsies by the ‘hot sodium hydroxide and tris’ (hotSHOT) method (Truett et al., 2000). The reactions determined the presence of both the neomycin phosphotransferase (NEO) gene and presence of either the D1 gene or the D2 gene. The following primers were used for the D1 reaction:

NEOf: 5'-CTG AAT GAA CTG CAG GAC GA-3',
NEOr: 5'-ATA CTT TCT CGG CAG GAG CA-3',
Drf: 5'-CAA TCA GAA AGT TCC TTT AAG ATG TCC-3',
Dr2: ATG GTG GCT GGA AAA CAT CAC AGC CCC-3',

and for the D2 reaction:

NEOf: 5'-CTT GGG TGG AGA GGC TAT TC-3',
NEOr: 5'-AGG TGA GAT GAC AGG AGA TC-3',
Drf: 5'-TGT GAC TGC AAC ATC CCA CC-3',
Dr2: 5'-GCG GAA CTC AAT GTT GAA GG -3'.

PCR products were separated on a 1.5% agarose gel. For D1 gene, amplification of a 172-bp PCR product indicated the presence of at least one transgenic allele and amplification of a 353-bp PCR product indicated the presence of at least one WT allele. For the D2 gene, amplification of a 280-bp band indicated the presence of at least one transgenic allele and a 105-bp band indicated the presence of at least one WT allele.

Instruments and procedures

Latent inhibition

Training and testing occurred in six identical conditioning chambers (21.6 cm × 17.8 cm × 12.7 cm, ENV-307W; Med Associates Inc., St Albans, VT, USA) housed in light and sound-attenuating boxes (ENV-022V, Med Associates Inc.). The conditioning chambers were composed of Plexiglas walls in the front and back, stainless steel sides, and a metal grid floor connected to a shock scrambler and generator. Each box contained a ventilation fan, mounted at the back to provide air exchange and background noise (69 dB white noise), and a sonalert mounted on the right wall for delivering the conditioned stimulus (CS) (85 dB) (ENV-323AW, Med Associates Inc.). Each chamber was equipped with a removable drink spout located in the left wall of the chamber. The lick spout was connected to a lickometer (ENV-250, Med Associates Inc.) which recorded the number of licks. The chambers were interfaced with a PC computer running MED-PC software (SOF-735, Med Associates Inc.) to control stimulus presentation and record data.

The LI protocol consisted of six stages: water restriction, pre-training, pre-exposure/no pre-exposure, conditioning, re-establishment of drinking, and testing.

Water restriction (days 1–7). Mice were placed on a 23-h water restriction schedule 7 d before pre-training began. Water in the test apparatus was given in addition to the daily ration of 1 h delivered in the home cages. This regimen was maintained throughout the experiment.

Pre-training (days 8–13). Mice were placed in the conditioning chambers and allowed to drink freely from a water sipper for 15 min. The number of licks made and latency to lick was recorded.

Pre-exposure (day 14). Mice were placed in the conditioning chambers without access to the water sipper. They were given 40 (expts 1 and 3), 60 (expt 2), 5 (expt 4) or 10 (expt 4) presentations of an 85-dB 5-s tone with a 15-s interstimulus interval. NPE control mice were placed in the chambers for the same amount of time but received no pre-exposures to the tone.

Conditioning (day 15). Mice were placed in the experimental chambers without access to the water sipper. After 5 min, two tone-footshock pairings were presented. Each tone (CS) was of 5-s duration and was followed by a 1-s 0.38-mA US footshock. There was a 2.5-min interval between pairings and mice remained in the chamber for 5 min following the second tone-footshock presentation.

Lick training (days 16–17). Mice were placed in the conditioning chambers for 15 min and given free access to the water sipper to re-establish licking in the chamber prior to testing. Mice that did not lick consistently were omitted from the experiment at this stage (n = 2 for expts 1–4).

Testing (day 18). Mice were placed in the conditioning chambers with access to the water sipper. The number of licks and the time to complete licks 80–90 (A) and 90–100 (B) were recorded electronically. After the first 90 licks, the tone CS was presented until the
mouse reached lick 100. The standard measure of conditioned suppression was the time taken to complete licks 90–100 in the presence of the CS. A suppression ratio (SR) was calculated according to the formula $A/(A+B)$ yielding a scale of 0–0.5. Low SR indicates good learning while high SR indicates poor learning of the association between the tone and footshock. LI is demonstrated as higher SR in the PE group compared to the NPE group.

**Spontaneous locomotor activity**

Spontaneous locomotor activity was recorded using the open-field test. The open-field apparatus consisted of six Plexiglas boxes measuring 30 cm × 17 cm with 25 cm walls. All walls were dark except for one transparent wall in each box allowing light to enter. Animals were placed in the boxes to habituate for 30 min. Twenty-four hours after the habituation session the animals were again placed into the open-field boxes for 30 min and total distance moved was recorded and analysed using a computerized video-tracking program EthoVision (version 3.1, Noldus, The Netherlands). Six animals were tested simultaneously in six separate open-field arenas. Between mice, all arenas were thoroughly cleaned with water and odour-free soap.

**Motor coordination**

The rotarod test was used as a measure of motor coordination. Animals were placed on a plastic rod rotating at 3 rpm (Rota-Rod/RS, LSI, Letica, Barcelona, Spain). Latency (s) to fall from the rod up to a maximum of 300 s was manually recorded. If a mouse fell within the first 5 s, the trial was restarted.

**Experimental procedure**

Experiments were run in the following order:

*Expt 1*

LI at 40 PE in $D_1$ KO mice ($n = 19$ WT, $n = 24$ $D_1$ KO). Mice were tested first on rotarod activity, 4 d later on locomotor activity and 14 d later began water deprivation schedule for LI. The LI procedure was run using 40 PE to produce low levels of LI in WT mice, this number was determined by pilot experiments in our laboratory.

*Expt 2*

LI at 60 PE in $D_1$ KO mice ($n = 33$ WT, $n = 25$ $D_1$ KO). The LI procedure was run using 60 PE to produce LI in WT mice.

*Expt 3*

LI at 40 PE in $D_1$ KO mice ($n = 24$ WT, $n = 21$ $D_1$ KO). Mice were tested first on rotarod activity, 2 d later on locomotor activity and 18 d later began water deprivation schedule for LI. The LI procedure was run using 40 PE.

*Expt 4*

This experiment was performed to further investigate LI in $D_1$ KO males as expt 3 showed that LI was still high at 40 PE in male $D_1$ KO WT mice. LI was tested at 5 PE and 10 PE in male $D_1$ KO mice ($n = 36$ male WT, $n = 15$ male $D_1$ KO) to produce low LI in WT mice.

**Statistics**

Statistics were performed using SPSS software, version 15 (SPSS Inc., Chicago, IL, USA). For LI experiments independent analysis of variance (ANOVA) was used with SR as dependent variable and exposure (NPE/PE) genotype (WT/KO) and sex (male/female) as factors. For post-hoc comparisons and other behavioural experiments Student’s independent $t$ tests were used. Five extreme values (>3 standard deviations of the mean) occurred in expt 3 and were removed from analysis: one WT PE female, one WT NPE female, one KO NPE male, two WT NPE males. No extreme values were found in any other experiment. Unless otherwise indicated, data were collapsed across sex when there was no effect of sex and no interaction between sex and exposure or genotype.

**Results**

**LI in $D_1$ receptor KO mice**

There was a clear enhancement of low LI (40 PE) in $D_1$ KO mice compared to WT littermates (Figure 1a). A two-way ANOVA with exposure, genotype and sex as factors demonstrated a significant effect of exposure $[F(1,34) = 6.113, p = 0.01]$, genotype $[F(1,34) = 6.812, p = 0.01]$ and a significant exposure × genotype interaction $[F(1,34) = 5.667, p < 0.05]$. No sex differences were found $[F(1,34) = 0.001, n.s.]$. Post-hoc $t$ tests confirmed that $D_1$ KO animals showed a significant difference between NPE and PE groups (denoting LI) whereas WT animals did not $[D_1$ KO NPE vs. $D_1$ KO PE: $t(21) = -2.853, p < 0.01$; WT NPE vs. WT PE: $t(14) = -1.139, n.s.]$. Moreover, PE groups were significantly different between genotypes whereas NPE groups were not $[WT$ PE vs. $D_1$ KO PE: $t(17) = 2.448, p < 0.05$; WT NPE vs. $D_1$ KO NPE: $t(18) = 1.856, n.s.]$. 

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post-hoc analysis the sexes were pooled. Post-hoc t tests confirmed an exposure effect (i.e. the presence of LI) in both genotypes [WT NPE vs. WT PE: t(28) = -2.545, p = 0.01; D2 KO NPE vs. D2 KO PE: t(19) = -2.409, p < 0.05]. Post-hoc t tests showed no differences between NPE groups or PE groups [WT NPE vs. D2 KO NPE: t(23) = 1.230, n.s.; WT PE vs. D2 KO PE: t(27) = 0.897, n.s.].

Baseline differences in drinking rates during pre-training between genotypes were not found in any experiment. Repeated-measures ANOVA on drinking rates in exp 1 with pre-training day and genotype as factors showed a significant effect of pre-training day [F(5, 35) = 4.840, p < 0.01], no effect of genotype [F(5, 39) = 0.123, n.s.] and no pre-training day × genotype interaction [F(1, 35) = 1.911, n.s.]. The same was found for exp 2: a significant effect of pre-training day was found [F(5, 52) = 8.446, p < 0.001], no significant effect of genotype [F(1, 56) = 0.136, n.s.] and no pre-training day × genotype interaction [F(5, 52) = 1.111, n.s.]. Similar results were found for expts 3 and 4 (data not shown). No baseline changes in drinking behaviour were seen in the test phase of the experiment either as measured by the time A scores. Expt 1: no effect of group [F(1, 34) = 1.580 n.s.], genotype [F(1, 34) = 0.710, n.s.], or sex [F(1, 34) = 0.470, n.s.]. Expt 2: no effect of group [F(1, 46) = 0.394, n.s.], genotype [F(1, 46) = 0.313, n.s.] or sex [F(1, 46) = 0.434, n.s.]. Expt 3: no effect of group [F(1, 33) = 0.641, n.s.], genotype [F(1, 33) = 1.742, n.s.] or sex [F(1, 33) = 1.373, n.s.]. Expt 4: no effect of group [F(1, 50) = 1.01, n.s.] or genotype [F(1, 50) = 1.05, n.s.]. No effect of sex or genotype was found for NPE control learning groups in any of the four experiments (all F values < 1).
Spontaneous locomotor activity and motor coordination in D2 receptor KO mice

Figure 3a shows that D2 KO animals are hypoactive compared to WT littermates \(F(1, 40) = 90.840, p < 0.001\) in the open-field test where spontaneous locomotor activity over 30 min was measured. A sex difference was also found \(F(1, 40) = 10.859, p < 0.01\), where males tended to be hyperactive compared to females. Post-hoc t tests showed significant sex differences between D2 KO animals [male D2 KO vs. female D2 KO: \(t(23) = 2.923, p < 0.01\)] but not between WT animals [male WT vs. female WT: \(t(17) = 1.927, \text{n.s.}\)]. The significant genotype effect was found in both males and females [male WT vs. male D2 KO: \(t(19) = 5.895, p < 0.001\]; female WT vs. female D2 KO: \(t(21) = 7.973, p < 0.001\)].

Figure 3b shows that D2 KO mice demonstrate impaired motor coordination as measured by the rotarod test when compared to WT littermates \(F(1, 42) = 12.328, p < 0.001\). No sex differences were found \(F(1, 42) = 0.969, \text{n.s.}\). Post-hoc t tests confirmed the genotype effect [male WT vs. male D2 KO: \(t(21) = 5.895, p < 0.001\); female WT vs. female D2 KO: \(t(21) = 7.973, p < 0.001\)].

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D1 receptor KO mice and LI

Figure 4a indicates slightly enhanced LI in D1 KO mice. There was a significant effect of exposure \(F(1, 67) = 7.223, p < 0.01\) and a significant effect of genotype \(F(1, 67) = 4.716, p < 0.05\), but no exposure x genotype interaction suggesting the effect is not
Post-hoc tests revealed no effect of exposure [female WT NPE vs. female D1 t time measured in the open-field test. The D1 sexes are hyperactive compared to WT littermate controls as motor coordination in either sex measured on the rotarod.

\[ p < 0.001 \] and a significant exposure interaction \[ F(2, 47) = 11.76, p < 0.0001 \], no effect of genotype \( F < 1 \), nor a genotype × exposure interaction \( F(2, 47) = 1.573, n.s. \) \( t \) test-confirmed presence of LI in WT at 10 PE [NPE vs. 10 PE (t = 7.9, p < 0.001)].

Figure 5. (a) Locomotor activity in D1 KO mice \( n = 10–12 \), (b) motor coordination in D1 KO mice \( n = 10–12 \). Columns represent mean ± S.E.M. *** \( p < 0.001 \) compared to wild-type (WT) littermate controls of same sex. D1 KO mice of both sexes are hyperactive compared to WT littermate controls as measured in the open-field test. The D1 KO had no effect on motor coordination in either sex measured on the rotarod.

To investigate further the suggestion from the above data that male D1 KO mice do not show enhanced LI a further experiment was performed investigating LI at very low numbers of exposures such that WT mice do not show LI. Figure 6 shows that at 5 PE where WT male mice do not show LI, there is similarly no LI in D1 KO male mice. Furthermore, at 10 PE there is a trend towards disruption in D1 KO mice. There was a significant effect of exposure \( F(1, 47) = 11.76, p < 0.0001 \), no effect of genotype \( F < 1 \), nor a genotype × exposure interaction \( F(2, 47) = 1.573, n.s. \) \( t \) test-confirmed presence of LI in WT at 10 PE [NPE vs. 10 PE (t = 7.9, p < 0.001)].

Spontaneous locomotor activity and coordination in D1 receptor KO mice

Figure 5a shows that in the open-field test of spontaneous locomotor activity, D1 KO animals are hyperactive compared to WT littermates with a significant effect of genotype \( F[1, 41] = 40.596, p < 0.001 \) and no effect of sex \( F[1, 41] = 0.471, n.s. \).

Figure 5b shows no effect on motor coordination in D1 KO mice of either sex. No effect of genotype

Figure 6. The effects of D1 KO on latent inhibition (LI) at 5 and 10 pre-exposure (PE) in wild-type (WT) male mice only \( n = 5–8 \). Columns represent mean ± S.E.M. ** \( p < 0.001 \) compared to no pre-exposure (NPE) for same genotype. WT mice show no LI at 5 PE but significant LI at 10 PE. D1 KO mice do not show LI at either 5 or 10 PE.

male WT NPE vs. male D1 KO NPE: \( t(15) = -0.789 \), n.s.] but a significant effect of exposure was found in WT but not D1 KO animals [male WT NPE vs. WT PE: \( t(26) = -3.099, p < 0.01 \); male D1 KO NPE vs. male D1 KO PE: \( t(7) = -1.515, n.s. \). This suggests potentiation of LI in female but not male D1 KO mice.

There was no significant effect of D1 genotype on baseline drinking rate. Repeated-measures ANOVA showed a significant effect of pre-training day \( F(5, 36) = 6.047, p < 0.001 \), no effect of genotype \( F(2, 40) = 2.236, n.s. \) and no genotype × pre-training day interaction \( F(10, 74) = 1.852, n.s. \).

To investigate further the suggestion from the above data that male D1 KO mice do not show enhanced LI a further experiment was performed investigating LI at very low numbers of exposures such that WT mice do not show LI. Figure 6 shows that at 5 PE where WT male mice do not show LI, there is similarly no LI in D1 KO male mice. Furthermore, at 10 PE there is a trend towards disruption in D1 KO mice. There was a significant effect of exposure \( F(1, 47) = 11.76, p < 0.0001 \), no effect of genotype \( F < 1 \), nor a genotype × exposure interaction \( F(2, 47) = 1.573, n.s. \) \( t \) test-confirmed presence of LI in WT at 10 PE [NPE vs. 10 PE (t = 7.9, p < 0.001)].
Discussion

These data demonstrate that D2 KO mice show markedly enhanced LI in low PE conditions where WT mice do not show LI. This profile reproduces the established profile of a range of antipsychotic drugs such as haloperidol, clozapine, remoxipride in LI in rats and mice (Mongeau et al., 2007; Moran et al., 1996; Moser et al., 2000; Trimble et al., 1997; Warburton et al., 1994; Weiner et al., 2003). These data for the first time confirm using a genetic approach, a specific role for the D2 receptor in the modulation of LI. Furthermore, our results suggest that the enhancement of LI characteristic of antipsychotics can be elicited by deletion of the D2 receptor subtype alone, and is therefore probably not dependent upon D3 and D4 receptors for which antipsychotic drugs also have affinity. In conditions where LI is present in controls, i.e. high number of pre-exposures (60 PE), D2 receptor gene deletion is without effect confirming that LI expression does not require the D2 receptor in conditions where WT mice show robust LI. This reproduces well-established effects of D2 receptor antagonists on LI (Moran et al., 1996; Ruob et al., 1998; Shadach et al., 2000).

It has been suggested that D2 KO mouse models possess limited value in modelling antipsychotic drug effects as, with the notable exception of side-effect-relevant behaviours such as motor deficits, they have not straightforwardly reproduced the behavioural effects of D2 antagonist antipsychotic drugs (Chen et al., 2006; Gainetdinov et al., 2001; Holmes et al., 2004; Waddington et al., 2005). For example D2 KO mice do not show similar effects to antipsychotics in sensory gating in the pre-pulse inhibition (PPI) model, although amphetamine abolition of PPI is prevented in these mice (Ralph et al., 1999). The finding of a similar profile of D2 receptor knockout and antipsychotic drugs in LI suggest that, for the constituent KO model, such a conclusion is premature. It is noteworthy that in the conditioned avoidance response model which is also sensitive to the effects of antipsychotic drugs, D2 KO mice similarly reproduce the effects of antipsychotic drugs, although sex differences in this model have not been addressed (Smith et al., 2002).

The present study also confirms that D2 gene deletion produces hypoactivity and impaired motor coordination as has been shown previously (Aoyama et al., 2000; Baik et al., 1995; Chausmer et al., 2002; Clifford et al., 2000; Fowler et al., 2002). This confirms that in this congenic line the established locomotor activity and motor coordination impairing profile of D2 antagonists is also reproduced (Aoyama et al., 2000).

In D1 KO mice we unexpectedly found significant enhancement of low LI in female but not male mice. However, at 40 PE in WT males there was a higher baseline LI than in the female WT cohort perhaps allowing enhancement to be seen more readily in D1 KO females. We have subsequently replicated this higher LI in male WT D1 mice at 20, 40 and 60 PE suggesting it is a robust effect (data not shown) and exp 4 has shown that we still see LI at 10 PE in male WT animals although this is not seen at 5 PE. We cannot fully explain this difference at present, however, it is possible that longer weaning in D1 WT mice may have exerted a beneficial cognitive effect on males as it is known that curtailment of weaning in male mice has negative behavioural consequences and induces corticosterone abnormalities (Wurbel and Stauffacher, 1997), this may also explain the baseline differences in WT activity and rotarod that we observed. It is clear that the amount of pre-exposure required to produce LI in WT animals differs between the sexes in these mice, with males showing no LI at 5 PE and females no LI at 40 PE. These differences are, however, consistent with studies of LI in humans where females show reduced LI compared to males (Lubow and De la Casa, 2002). Despite this higher baseline in males there is still a considerable margin to detect enhancement of LI if it existed and we see no evidence for this. At 5 PE we see no LI in either WT males or D1 KO males and a trend towards reduced LI in D1 KO males at 10 PE. This supports the suggestion that D1 KO males do not show enhanced LI compared to WT males. Further studies using larger cohorts than used in the present study would be desirable before concluding definitively that D1 KO males do not show enhanced LI under any experimental conditions. At 10 PE there is some suggestion that D1 KO males may show a deficit in LI produced by 10 PE, although this is not significant in the present study. This possibility will need to be replicated and extended to larger cohorts in follow-up studies. These data nonetheless suggest that D1 receptor deletion may enhance LI exclusively in females. This is contrary to prior studies in rats showing no effect of D1 antagonists on low LI (Trimble et al., 2002). It is notable that these studies in common with the majority of behavioural studies in rodents were carried out exclusively in males (Hughes, 2007).

D1 KO mice showed hyperactivity and no deficits in rotarod motor coordination that was similar between
the sexes confirming previous studies in these mice (Holmes et al., 2004). The lack of a similar profile of sex difference in activity and motor coordination additionally suggests that the LI sex difference we have identified is highly specific to LI and not attributable to generalized behavioral/motor compromise in these mice. This is further supported by the fact that there is no effect of genotype or sex on the NPE learning groups.

Clinical trials reporting no benefit of D₁ antagonists in schizophrenia patients have been primarily carried out with male patients (de Beaurepaire et al., 1995; Den Boer et al., 1995). The present data might help to explain this lack of efficacy. These data indicate that enhancement of LI may involve differential regulation by D₁ and D₂ receptor subtypes in males and females.

The existence of sex differences in phenomenology, epidemiology, neuroanatomy and response to antipsychotic drugs in schizophrenia has been well described (Goldstein et al., 2002; Lewine et al., 1996; Usall et al., 2007). Recently, Rubin et al. (2008) found sex differences in cognitive responses to antipsychotic drugs in patients with schizophrenia. The present results may help to explain sex differences in response to antipsychotic drugs in terms of dissociable dopaminergic substrates; one possible mechanism is an interaction with sex hormones. It is well established that the oestrous cycle influences variation in basal extracellular concentration of striatal DA, both in drug-stimulated DA release and in DA-mediated behaviours (Becker, 1999). Oestrogen directly acts on the striatum and nucleus accumbens to enhance DA release and DA-mediated behaviours (Le Saux et al., 2006).

High levels of oestrogen have been shown to increase mesolimbic DA transmission (known to disrupt LI) (Gray et al., 1995, 1997, 1999; Joseph et al., 1993; Van Hartesveldt and Joyce, 1986) while ovariectomy reduces basal extracellular DA, amphetamine-induced striatal DA release, and behaviours mediated by the striatal DA system (Becker, 1999).

Sex differences have also been reported in rodents in behavioural response to drugs of abuse such as cocaine, ethanol and MDMA; these differences have been shown to be mediated in part by the D₁ receptor (Ferris et al., 2007; Festa et al., 2006; Melnick and Dow-Edwards, 2001). Behavioural responses to D₁ agonists and antagonists have also been demonstrated to differ between the sexes (Nazarian et al., 2004).

Sexually dimorphic behavioural phenotypes in mice with mutations for genes mediating diverse aspects of DA neurotransmission have also been reported including DARPP-32 and catechol-O-methyltransferase (COMT) function (Babovic et al., 2007; Harrison and Tunbridge, 2007; Waddington et al., 2005).

In all KO mouse studies where the genetic deletion is present from birth there is the potential for compensatory developmental changes to occur. While this cannot be excluded here, this is currently the only approach to differentiate the impact of the absence of specific DA receptor subtypes in isolation. The recent development of inducible D₁ mutants will be of value in future exploration of sexually dimorphic contributions of different DA receptor subtypes to salience allocation processes and their aberrant states in psychosis (e.g. Gantois et al., 2007). The present results are, however, consistent with predictions from pharmacological studies in LI for the D₂ receptor, while the novel sex differences for D₁ suggested will generate further investigation using pharmacological approaches and a wider range of behavioural models known to be sensitive to antipsychotic drug effects.

In summary we have shown that the antipsychotic-like profile of enhanced LI can be elicited by D₂ but not D₁ receptor KO in males but by both D₁ and D₂ receptor KO in females. This suggests the possibility that the mechanism of action of antipsychotic drugs may differ between the sexes and that the D₁ receptor may be an important factor in dissociable male and female responses to antipsychotic drugs.

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Statement of Interest

None.

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